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3 Sub-acute Toxicity Study of The Ethyl Acetate Fraction of Asam Kandis Rinds (*Garcinia cowa* Roxb.) on the Liver and Renal Function in Mice

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ABSTRACT

Objective: The present study investigated the sub acute toxicity of the ethyl acetate fraction of asam kandis (*Garcinia cowa* Roxb) Rinds in mice. **Material and Methods:** Sub acute toxicity study was carried out by giving orally at dose 500, 1000 dan 2000 mg / kgBW extract to five mice at 21 days. Animals were observed individually for any clinical signs of toxicity or mortality for 14 days. Measured parameters were SGPT levels, serum creatinine levels, weight ratio of liver and kidney. Extract was given orally at dose 500, 1000 and 2000 mg/kgBW for 21 days. Observations were done on day 8th, 15th and 22th using blood serum, liver and kidneys of mice. Data were analyzed by using two-way ANOVA followed by Duncan's Multiple Range Test. **Results:** The ethyl acetate fraction of *G. cowa* at doses 500, 1000 and 2000 mg/kgBW gave significant effect on increasing SGPT levels and decreasing levels of serum creatinine ($p < 0.05$). The length of treatment gave significant effect on decreasing levels of serum creatinine, weight ratio of liver and kidney ($p < 0.05$). **Conclusion:** The dosage of the ethyl acetate fraction of asam kandis rinds provides significant effect on the SGPT and serum creatinine levels of male white mice. The duration of administration of ethyl acetate fraction of asam kandis rinds provides significant effect on serum creatinine levels, the weight ratio of liver and kidney organ of male white mice.

Key words: Sub-acute toxicity, *Garcinia cowa* rinds, SGPT, Creatinine serum, Weight ratio of liver and kidney.

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INTRODUCTION

Garcinia (Guttiferae) species are commonly found in the lowland areas of the rainforests. They consist of about 400 species concentrated mainly in southeast Asia.¹ *Garcinia* species are typically small to medium evergreen fruit trees, and usually produce hard timber. Traditional uses and medicinal properties of this species were documented by Burkill.²

The chemical constituents and biological activity of *Garcinia* (Guttiferae) has been extensively investigated. This genus is reported to contain quinone,³ xanthenes,^{3,4} benzophenones,⁵ triterpenes,⁶ biflavonoids⁷ and benzoquinone.⁸ The pharmacological activities of this genus were anticancer,^{9,10} anti-inflammatory,¹¹ antibacterial,¹² antiviral,¹³ antifungal and antioxidants.¹⁴

G. cowa, commonly known as asam kandis in Indonesia, is widely distributed throughout Indonesia, Malaysia, Thailand and Myanmar. Many parts of *G. cowa* have been used in traditional folk medicine. For example, the bark, latex and root have been used as an anti fever agent^{15,16} while the fruit and leaves have been used for indigestion and improve flow of blood circulation, and as an expectorant.¹⁶ The chemical constituents and biological activity of various parts of *G. cowa* have been intensively investigated. The major components found were xanthenes and qu-

none.³ Currently, 12 compounds have been isolated from the bark,¹⁷ leaves¹⁸ and root.¹⁹

Previous studies showed that the ethyl acetate fraction of rinds of asam kandis (*G. cowa*) have a cytotoxic effect on HeLa cervical cancer cells with IC₅₀ value of 16.194 ± 3.5019 mg / mL. This fraction is potential to be developed as a new source in developing cancer drugs. Further research is still needed to determine the level of safety in animal experiments. Safety evaluation of the use of traditional medicine should be carried out include the acute toxicity test, sub-acute toxicity test, sub-chronic toxicity test and chronic toxicity test.²⁰ This study conducted sub-acute toxicity tests which aims to determine the safety of the ethyl acetate fraction of asam kandis rinds with determine the toxic effects of chemicals which given repeatedly that potentially in target organs, determine dose-response relationships for the range of the dose and exposure time, as well as for evaluated the maximum dose that does not cause toxic effects on repeated administration.²⁰

Liver and kidney function is one of the observations that doing at this toxicity test. Liver is the organ that plays a role in the function of metabolism and excretion from the body. Toxic effects of the drugs are often seen in the liver, because liver metabolizes all drugs and foreign substances that enter the body.

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Liver disorders characterized by elevated serum transaminase activity such SGPT (Serum Glutamic Pyruvic Transaminases) in serum.²¹ Kidney also the main target organ of the toxic effect, because its produce urine which is the main route of excretion toxicant and has a high volume of blood flow. One indicator of kidney damage is an increase or a decrease in creatinine levels in the body.²²

MATERIALS AND METHODS

Chemicals and Reagents

Assay kits for kidney and liver function indices were products of Randox Laboratories limited, United Kingdom. Other chemicals and reagents were all of analytical grade.

Plant Collection, Authentication and Extraction

Fresh rinds of *Garcinia cowa* were collected from Batu Busuk, Padang, West Sumatra, Indonesia in August, 2015. The plant was identified and authenticated at the Herbarium Andalas University (ANDA) and was assigned a voucher number FSW-001 after which a voucher specimen was prepared and deposited at the University Herbarium. Fresh Rinds of *G. cowa* were then chopped into small pieces, air-dried at room temperature for 10 days to a constant weight and subsequently pulverized into fine powder. Powder sample (500 g) was soaked in 4 liters of 70% ethanol for 24 hours. The extract was filtered (with Whatman No. 1 filter paper) and the resulting filtrate was concentrated with a rotary evaporator (40°C). After that, the product was lyophilized to give 12.0 g of residue, according to a yield of 2.4%. This was then stored in a desiccator for further use.

Experimental Design

Healthy Swiss albino mice, 6-8 w old, of either sex, having body weights in the range of 20 ± 3 g were procured from the animal house of Faculty of Pharmacy Andalas University. They were randomly divided into different experimental groups. Animals were acclimatized to laboratory conditions for at least one week prior to the start of the experiment. They were provided with commercial food pellets and water ad libitum unless stated otherwise. The study was approved by the Ethics Committee Faculty of Medicine Andalas University.

Sub-acute toxicity study

The sub-acute toxicity study was performed according to OECD guideline 407. A number of 40 mice were divided into 4 groups. Group I (control group) received a solution of 5% tween 80, group II-IV were orally administrated with the ethyl acetate fraction of *G. cowa* rinds with dose 500 mg/Kg BW, 1000 mg/Kg BW, and 2000 mg/Kg BW respectively once a day for 7 days, 14 days and 21 days. The level of creatinine in serum and SGPT was measured on the 8th day, 15th day and 22nd day. Body weight of animals as well as their food and water consumption was recorded weekly during the study period. The animals were observed signs of intoxication and death during the trial period. The blood samples of all the animals were taken prior to necropsy. The weight ratio of liver and kidney were determined by comparing the weight of the liver and kidneys of mice against weight gain in mice. The data were analyzed by statistical two-way ANOVA followed by Duncan's Multiple Range Test.

RESULTS

SGPT levels of mice was not significantly affected by the duration of administration ($p > 0.05$), but were significantly affected by the dosage ($p < 0.05$). There was no significant effect between duration of administration and the dosage on the SGPT levels ($p > 0.05$) (Table 1).

Creatinine levels of mice were significantly affected by the duration of administration and dosage ($p < 0.05$). There is a significant effect of interaction between duration of administration and dosage on the serum creatinine level ($p < 0.05$) (Table 2 and Figure 1).

The weight ratio of liver were significantly affected by the duration of administration ($p < 0.05$), but was not significantly affected by the dosage ($p > 0.05$). There is no interaction effect between duration of administration and dosage ($p > 0.05$) (Table 3 and Figure 2).

The weight ratio of kidneys were significantly affected by the duration of administration ($p < 0.05$), but was not influenced significantly by the dosage ($p > 0.05$). There was no significant effect on the interaction between duration of administration and dosage ($p > 0.05$) (Table 4 and Figure 3).

Table 1: The effect of dosage and duration of administration of the ethyl acetate fraction of asam kandis rinds on SGPT level

Dosage	Average levels of SGPT (U/L)			Average levels of SGPT (U/L)
	8 th day	15 th day	22 nd day	
Control \pm SD	27.50 \pm 3.235	37.74 \pm 0.933	36.66 \pm 13.074	33.98 \pm 5.628
500 mg/kg BW \pm SD	38.82 \pm 2.801	32.89 \pm 6.537	37.20 \pm 9.805	36.30 \pm 3.066
1000 mg/kg BW \pm SD	37.20 \pm 1.617	47.99 \pm 7.644	37.74 \pm 3.367	40.20 \pm 6.076
2000 mg/kg BW \pm SD	52.30 \pm 13.565	51.22 \pm 4.070	40.98 \pm 7.293	48.17 \pm 6.249
Average levels of SGPT (U/L)	41.17 \pm 10.982	39.90 \pm 7.887	38.01 \pm 1.993	

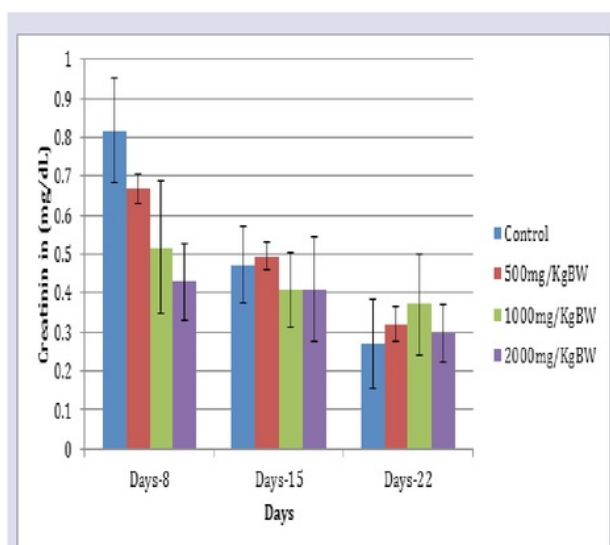


Figure 1: The effect of ethyl acetate fraction of asam kandis rinds on serum creatinine levels of white males mice

Table 2: The effect of dosage and duration of administration of ³ ethyl acetate fraction of asam kandis rinds on creatinine level

Dosage	Average levels of creatinine (mg/dL)			Average levels of creatinine
	8 th day	15 th day	22 nd day	
Control \pm SD	0,82 \pm 0,134	0,47 \pm 0,098	0,27 \pm 0,113	0,52 \pm 0,276
500 mg/kgBW \pm SD	0,667 \pm 0,037	0,494 \pm 0,037	0,32 \pm 0,043	0,49 \pm 0,173
1000 mg/kgBW \pm SD	0,52 \pm 0,170	0,41 \pm 0,098	0,37 \pm 0,128	0,43 \pm 0,076
2000 mg/kgBW \pm SD	0,43 \pm 0,098	0,41 \pm 0,134	0,30 \pm 0,074	0,38 \pm 0,072
Average levels of creatinine	0,61 \pm 0,171	0,45 \pm 0,044	0,32 \pm 0,042	

Table 3: The effect of dosage and duration of administration of ³ ethyl acetate fraction of asam kandis rinds on weight ratio of liver

Dosage	Average weight ratio of liver			average weight ratio of liver \pm SD
	8 th day	15 th day	22 nd day	
Control \pm SD	0,061 \pm 0,009	0,046 \pm 0,009	0,042 \pm 0,002	0,049 \pm 0,01
500 mg/kgBW \pm SD	0,057 \pm 0,013	0,0399 \pm 0,004	0,048 \pm 0,006	0,048 \pm 0,008
1000 mg/kgBW \pm SD	0,06 \pm 0,008	0,051 \pm 0,01	0,047 \pm 0,007	0,053 \pm 0,006
2000 mg/kgBW \pm SD	0,063 \pm 0,019	0,045 \pm 0,003	0,046 \pm 0,003	0,051 \pm 0,01
average weight ratio of liver \pm SD	0,060 \pm 0,002	0,045 \pm 0,004	0,046 \pm 0,003	

Table 4: The effect of dosage and duration of administration of ³ ethyl acetate fraction of asam kandis rinds on weight ratio of kidneys

Dosage	average weight ratio of kidney			average weight ratio of kidney
	8 th day	15 th day	22 nd day	
Control \pm SD	0,017 \pm 0,0009	0,012 \pm 0,0003	0,012 \pm 0,0004	0,014 \pm 0,003
500 mg/kgBW \pm SD	0,015 \pm 0,0006	0,012 \pm 0,0003	0,012 \pm 0,0006	0,013 \pm 0,008
1000 mg/kgBW \pm SD	0,015 \pm 0,0014	0,012 \pm 0,0009	0,013 \pm 0,0012	0,013 \pm 0,002
2000 mg/kgBW \pm SD	0,017 \pm 0,0032	0,012 \pm 0,0006	0,013 \pm 0,0013	0,014 \pm 0,003
average weight ratio of kidney	0,016 \pm 0,0012	0,012 \pm 0,0003	0,013 \pm 0,00048	

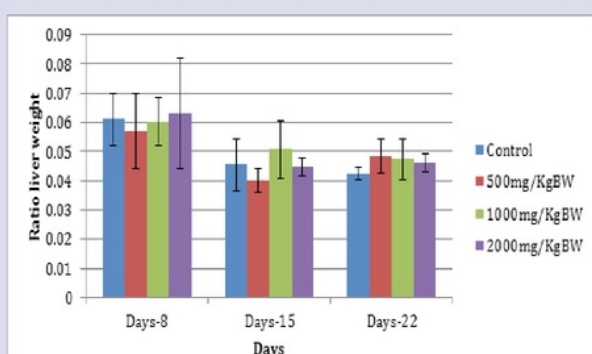


Figure 2: The effect of ethyl acetate fraction of asam kandis rinds on ratio of liver weight of white males mice.

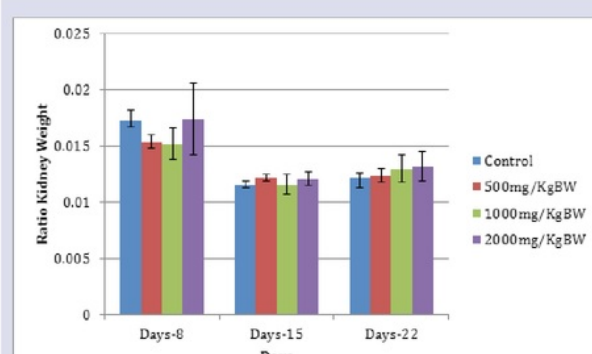


Figure 3: The effect of ethyl acetate fraction of asam kandis rinds on ratio of kidney weight of white males mice.

DISCUSSION

Decreased levels of SGPT on 8th day, 15th day and 22nd day is not much different. Decreased levels of SGPT caused by the ability of all cell to be regenerate. Cell regeneration is the process of formation of cells to replace the dead cells. Liver cells having a stable cell. It is estimated that within 5 days the mice cells had regenerated, thus causing a decrease in SGPT levels.²² This is the underlying reason for a decline in SGPT levels at 15th day and 22nd day. The average levels of SGPT at dose 2000 mg/kgBW is higher than the control group, its caused by damage and lysis that happen in liver cells, so SGPT enzyme that located in the cytoplasm will come out and get into the serum, which will increasing SGPT levels. The results of the study did not find any damage to the kidneys of mice. In general, decreased levels of creatinine happen in each group, but still within the normal range of creatinine levels in mice (0.2 to 0.9 mg / dL).²³ The average weight ratio of liver decrease occur in 15th day and 22nd day. It is due to the recovery process by liver cells, because liver cells has the ability to regenerate their cells, which is a process of formation of new cells to replace dead cells.⁷ Liver which exposed with toxicant will lead to death cell. Based on morphology, death of liver cells has two mechanisms, namely, necrosis and apoptosis. Necrosis is the process of death cell that is marked by swelling of cells and release of cell contents. Apoptosis is a process of death cell characterized the occurrence of cell shrinkage, chromatin condensation, nucleus fragmentation, formation of apoptotic bodies and usually do not cause inflammation.⁷ The average weight ratio of liver increased in line with the increase in dose, it can be caused by inflammation and necrosis of the liver cells

A high ratio of kidney on 8th day due to edema or swelling that occurs in kidney. Edema that occurs due to the decrease in blood pressure, thus resulting renin that will change angiotensin I became angiotensin II, resulting in vasoconstriction of the blood vessels, improves water retention, and increased plasma volume. Edema can be caused by a disturbance in glomerular membrane permeability, so that the fluid balance will be disturbed and moved to extracellular.⁹

CONCLUSION

The dosage of the ethyl acetate fraction of *G. cowa* rinds provide significant effect on the SGPT and serum creatinine levels of male white mice ($p < 0.05$). The duration of administration of ethyl acetate fraction of asam kandis rinds provides significant effect on serum creatinine levels, the weight ratio of liver and kidney organ of male white mice ($p < 0.05$)

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CONFLIC OF INTEREST

None

ABBREVIATIONS USED

SGPT: Serum Glutamic Piruvic Transaminases; ANOVA: Analysis of variance; OECD: Organisation for Economic Cooperation and Development.

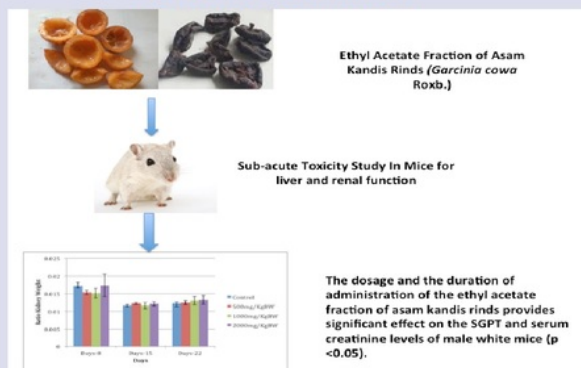
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PICTORIAL ABSTRACT



SUMMARY

- The ethyl acetate fraction of asam kandis rinds provides significant effect on the SGPT and serum creatinine levels of male white mice. The duration of administration of ethyl acetate fraction of asam kandis rinds provides significant effect on serum creatinine levels, the weight ratio of liver and kidney organ of male white mice.

ABOUT AUTHORS



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Nelsi Fitri Hayaty: She is undergraduate student from Andalas University. Her research is sub acute toxicity studies of *Garcinia cowa*



Dian Ayu Juwita: She is a young lecturer from Faculty of Pharmacy Andalas University. Her research is toxicity studies of Sumatran plants



Almahdy: Got his undergraduate degrees from Andalas University and finished Her PhD from Andalas University in 2006. He is positioned as Professor of Pharmacy at Faculty of Pharmacy, Andalas University. His research is in toxicology and biological activity studies of Sumatran Plants

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